*Br. J. Cancer* (1984) **49**, Suppl. VI, 81–90

# The DNA damage-repair hypothesis in radiation biology: Comparison with classical hit theory

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**Summary** In classical theories of radiobiological action, cell killing is viewed as an inevitable consequence of the accumulation of some given number of physical "hits" in sensitive, intracellular targets. Shoulders on survival curves are attributed to the need for more than one hit to produce the observed effect, and to the random distribution of these hits among the cells in an irradiated population. Such curves start with zero slope at very low doses, and, at high doses, they approach, asymptotically, exponential slopes that are inversely proportional to the dose required for one hit, or to inactivate a single target. Unfortunately, these simple ideas provide no credible explanation for the dramatic changes in apparent final slope, and the total abolition of shoulders, that are observed in many radiation-sensitive mutants.

The damage-repair hypothesis asserts that the surviving fraction of cells in a mutagen-treated population is proportional to the number of potentially lethal lesions that are not removed by any repair process. Evidence indicates that these repairable lesions are located in DNA; however, this fact is irrelevant to the mathematical development of dose-response equations under the damage-repair hypothesis. The survival curves for repair-proficient cells generally exhibit a shoulder which reflects a decline in the efficiency of repair with increasing dose. Introduction of the concepts of "error-prone" and "recombinagenic" repair allows the extension of these ideas to data on induced mutation and mitotic recombination.

In the early decades of this century three surprising observations on the genetic action of ionizing radiation attracted the attention of physicists and led to the development of physico-mathematical theories for their explanation. These were, first, the remarkable energetic effectiveness of X-rays in producing biological effects; second, the apparent lack of threshold dose levels, well-known for common poisons, for both cell killing and mutagenesis; and third, the remarkable stability of genes toward mutation, except for those induced by X-rays and ultraviolet light, together with an anomalously high temperature coefficient ($Q_{10}$) for spontaneous mutation. In all of these respects the effects of radiation on cells were seen to differ dramatically from those of ordinary chemicals.

The first two observations stimulated the development of the hit and target theories for radiobiological actions, according to which the mathematical form of dose-response curves for killing and mutation was attributed to the discontinuous, random, nature of the highly energetic X-ray absorption events in matter (Timoféeff-Ressovsky & Zimmer, 1947; Zimmer, 1961). On this basis the shapes of survival and mutation frequency curves were considered to reflect primarily the statistics of radiation absorption events, rather than the biochemistry of the irradiated cells.

The third observation led Delbrück to propose that genes must have extraordinarily stable molecular structures, with anomalously high energy thresholds for mutagenic transitions, which could, however, be breached by the high quantum energies of X-ray photons (Timoféeff-Ressovsky *et al.*, 1935). On this view one would not expect chemicals to be mutagenic, which seemed to be the case in 1935 (Auerbach, 1973).

The well-known post-war developments in molecular genetics have made these simple physical ideas untenable. The genome is now known to be a remarkably "fluid" macromolecular structure, and the physical basis of genetic stability and change depends on an amazing battery of enzymes and other proteins, of widely varying activities and specificities, involved in various aspects of DNA turnover and metabolism. In particular, it has become clear that cellular sensitivity to killing and mutation by radiation reflects far more the biology of the cells themselves, than the physics of X-ray absorption, although certainly physics does play a significant role, especially in the effects of densely-ionizing radiations (Blakely *et al.*, 1979, and references therein).

Central to the development of these new views was the discovery, by radiation biologists, of various modes of DNA repair, and their role in the inactivation and recovery of microorganisms exposed to radiation or chemical mutagens (Haynes, 1964; Hanawalt *et al.*, 1979; Haynes & Kunz, 1981). In this paper, we outline the "DNA damage-repair" hypothesis and show how it can be used in the interpretation of the shapes of dose-response curves for the lethal and mutagenic effects of various physical and chemical agents (Haynes, 1966; Haynes & Eckardt, 1980; Wheatcroft *et al.*, 1975). These ideas can be regarded as a biological re-interpretation of physical hit theory, and so we will be at pains to point out some of the fundamental differences between the classical theories and "repair" theory. Unfortunately, these latter-day concepts no doubt still over-simplify the underlying biochemical complexity of cellular responses to mutagenic agents. It must also be emphasized that although repair theory provides a useful framework for thinking about the observed phenomena, explicit molecular mechanisms cannot be deduced from kinetic analysis of dose-response data alone, and such data often are inadequate even to distinguish among different possible mechanisms. However, to be acceptable, proposed molecular mechanisms for cell killing and mutagenesis must be consistent quantitatively with observed dose-effect relations. In all of this it is reassuring to bear in mind the old adage that theories are obliged to be useful, even if they are not true.

### *Salient features and difficulties of hit/target theories*

In classical hit theory it is assumed that cell killing or mutation is a direct and inevitable consequence of the accumulation of some minimal number ($n$) of physical "hits" in the cell. For ionizing radiation, these "hits" are identified with ionization absorption events and no allowance is made for any subsequent biochemical modification or repair of the initial radiochemical lesions. Hits are considered to be distributed randomly in uniformly irradiated, homogeneous, cell populations. Thus, surviving fractions and mutation frequencies are calculated by application of Poisson statistics to the average number of relevant physical hits per cell expected at any given dose. In the simplest "single-hit" cases, the predicted dose-response relations are exponential for cell survival and linear for mutation frequency (Zimmer, 1961; Haynes & Eckardt, 1980). If more than one hit is required to produce the observed effect, then, by definition, there is a threshold for the biological response at the level of the individual cell. However, the observed dose-response curves do not exhibit a discontinuous threshold response, but rather bend smoothly away from the origin, because of the random distribution in the number of hits per cell in the irradiated population: even at low doses there will be some "unlucky" cells that receive $n$ or more hits, and at high doses some "lucky" cells will escape being hit. Thus, in multihit cases, survival curves have smoothly bending shoulders with zero initial slope, and mutation frequency curves rise, again with zero initial slope, as the square or some higher power of dose depending on the value of $n$. Because of the mathematical nature of the Poisson distribution for $n>1$, multihit survival curves do not have terminal exponential slopes at finite doses but rather approach asymptotically the exponential slope for the single-hit response (Fowler, 1964).

Target theory is similar to hit theory except for the additional assumption that cells contain sensitive "target" structures within which the physical hits must occur to be relevant to the measured endpoint (Zimmer, 1961). A large number of models can be generated by assuming various numbers of targets, of possibly differing sensitivities, each requiring different numbers of hits to be affected. Multitarget models again predict zero initial slopes for dose-effect curves unless further complicating assumptions are made (Alper, 1979). Multitarget survival curves have an exponential final slope whose magnitude is proportional to the assumed target volume or cross-section.

The dose-response equations of classical hit/target theory often can be used to obtain satisfactory mathematical fits to radiobiological data. However, it is now clear that they present so many interpretive difficulties and practical shortcomings that their usefulness, both for empirical curve-fitting, and as a guide to further experiment, may be seriously questioned.

Despite experimental difficulties in making accurate measurements, the zero initial slopes that are required by multihit and multitarget models are not always observed, and shouldered survival curves often appear to have non-zero initial slopes (Alper, 1979; Leenhouts & Chadwick, 1978). It is also difficult to avoid the conclusion that many survival curves exhibit terminal exponential slopes in the biological dose range (Alper, 1979); this is inconsistent with simple multihit models. Such slopes can be accommodated more readily by multi-target theory, but since, on such models, they are considered proprotional to the sizes of the relevant intracellular targets it is difficult to see how they can be changed as greatly as they are by point mutations in radiation sensitive mutants (Figure 1).

There also are a number of more fundamental theoretical difficulties with classical hit theory. The

most important of these concerns the definition of "hits" as purely physical events whose number in the cell or target volume, at all times after irradiation, is assumed to be strictly proportional to dose (Zimmer, 1961). Such an assumption does not take cognizance of any possible biochemical modification of the initial radio-chemical lesions during expression of the biological endpoint. Furthermore, such physical hits, expressed as ionizations or excitations per unit volume, are not directly measured or observed experimentally, but rather their number is calculated from microscopic assumptions regarding radiation absorption processes in matter. The probability of survival is then computed by application of the Poisson distribution to the expected number of these physical hits at any given dose.

This classical approach is unsatisfactory because what actually is observed is a *biological* event, cell survival or mutation, together with a macroscopically uniform dose to the cells. Thus, from the experimental standpoint, it is the occurrence of survival or mutation that we observe as being randomly distributed among the cells in the irradiated population. In computing their probabilities, the Poisson distribution should be applied to the observed, randomly distributed, *biological* events, rather than to unobserved physical events. This is the viewpoint adopted in "repair" theory. The biological events are called lethal hits, or mutational hits. Such biological hits are measured directly in terms of surviving fractions, or mutation frequencies, respectively: one biological hit is said to have occurred per cell, on average, at a dose that leaves a fraction $e^{-1}$ of cells unaffected with respect to the endpoint measured (Haynes, 1966; Haynes & Eckardt, 1980). No such explicit distinction between physical and biological hits is made in classical theory for the very simple reason that they are assumed to be identical: the observed biological effects are considered to flow directly and inevitably from the initial physical hits.

*Salient features of repair theory*

It is in drawing this distinction between physical and biological hits that "repair" theory departs most significantly from classical hit theory. For our purposes, a physical hit is a potentially lethal, or premutational, lesion in whatever cellular targets are relevant to the endpoint assayed. Complex physicochemical mechanisms are involved in the formation of these lesions, whereas different, but equally complex biochemical and physiological processes are involved in the conversion, by the cell, of physical lesions to biological hits. Henceforth, we will use the word hit only in the biological context of lethal or mutational hits. In repair theory, lethal hits are assumed to be unrepaired lesions in DNA (Haynes, 1966). Mutational hits are assumed to arise from premutational lesions that have not been removed by "error-free" repair, but have been acted upon, or by-passed, by "error-prone" repair processes. On this basis it is possible to derive mathematically an even more diverse array of dose-response equations than is the case for the classical hit or target theories (Haynes & Eckardt, 1980).

*Examples of dose-response relations in UV-irradiated yeast*

Ionizing and ultraviolet radiations, and many chemicals, are capable of inducing cell killing, mutation and various recombinational events in yeast and, of course, other organisms as well (Haynes & Kunz, 1981; Kunz & Haynes, 1981). Sensitivity to these effects, and the detailed shapes of the dose-effect curves are strongly dependent on the genetic constitution of the cells and their physiological state before, during and after exposure. Exponential and shouldered survival

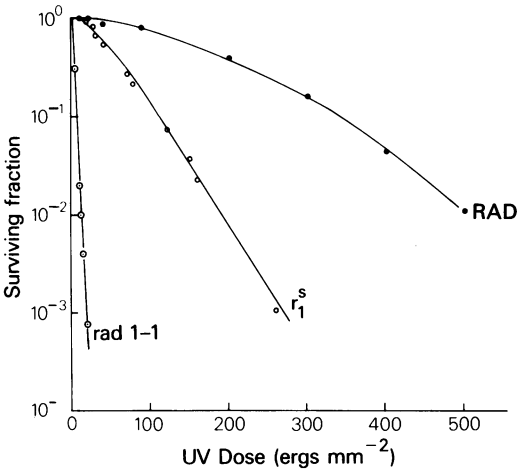


**Figure 1** UV survival for 3 related strains of haploid yeast. The *RAD* strain is wild-type for all known modes of DNA repair in yeast (over 90 genetic loci affect radiation sensitivity and/or DNA repair in this organism). The *rad* 1-1 strain is deficient in UV-induced pyrimidine dimer excision, however this strain has wild-type sensitivity to X-rays. The molecular basis for the sensitivity of strain $r_1^s$ hot yet been worked out. The *rad* 1-1 curve is exponential, a feature shared by most non-leaky repair mutants in yeast. The *RAD* curve has a zero initial slope, whereas for $r_1^s$ it may be non-zero. These data indicate the dramatic effects that repair deficiencies can have on sensitivity and survival curve shape. Similar phenomena have been observed also for X-rays and many chemical mutagens (Haynes & Kunz, 1981).

curves, together with almost every other conceivable type, have been observed for various mutagenic agents. The fact that certain single-gene mutations, now known to produce deficiencies in DNA repair, can alter drastically the magnitudes of both shoulders and apparent final slopes of survival curves (Figure 1) means that these curve parameters must be related to the gene functions blocked in the mutant strains. This conclusion seems to hold not only for ionizing and ultraviolet radiations, but for many chemical mutagens as well.

A similarly bewildering variety of induced mutation and recombination frequency (mutants or recombinants per survivor) curves have been reported in various systems. Depending on the assay employed, these curves may be linear, non-linear or multiphasic; they may also increase at powers of dose less than one, or possess maxima. And, most importantly, both elevated and depressed levels of forward and reverse mutation, and mitotic recombination, have been observed in strains known biochemically to be deficient in various modes of DNA repair. Examples of some of these diverse dose-response patterns for UV-irradiated yeast are given in Figures 2 and 3. The non-linear characteristics of these various curves can be attributed to the existence of DNA repair mechanisms whose efficiency is dose-dependent, and/or to some interdependence of the lethal and mutational responses (Eckardt & Haynes, 1977). It is often difficult to determine from which of these latter sources any particular type of non-linearity arises. To complicate matters further, population heterogeneity among the irradiated cells, as well as genuine target multiplicity, can be further sources of non-linear behaviour.

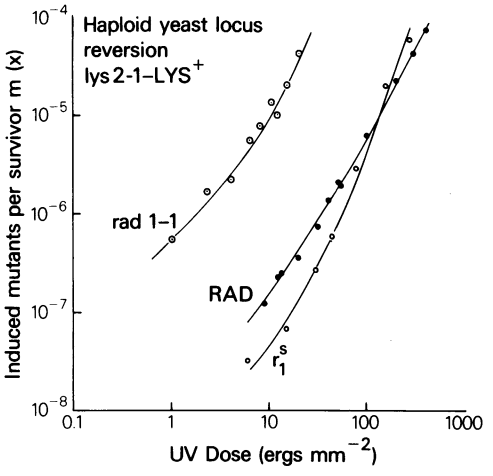


**Figure 2** Mutation frequency curves for reversion to lysine prototrophy in the same 3 strains of yeast as those illustrated in **Figure 1.** The data are plotted on double-log paper in order to illustrate the power of dose by which frequency increases: linear at low doses but non-linear at higher doses. In the *rad* 1-1 strain this "positive" departure from linearity at high doses can be attributed to stochastic dependence of mutation and killing ($\delta$-effects) (Eckardt & Haynes, 1977). In the *RAD* strain, the non-linearity appears to be associated with the UV-induction of error-prone repair. The fact that the curve for the *rad* 1-1 mutant lies above that for *RAD* wild-type, indicates that relevant UV-induced pre-mutational lesions are not repaired in the former strain but are in the latter. It would appear from this data that pre-mutational lesions also are repaired in $r_1^s$ even though it is deficient in some mode of repair.

*Error-free and error-prone repair or bypass of DNA damage*

The DNA damage-repair hypothesis, as applied in the interpretation of dose-effect curves for cell killing and mutation, is summarized schematically in Figure 4. The left-hand panel illustrates the shouldered and exponential survival curves often observed for isogenic cells that differ only in DNA

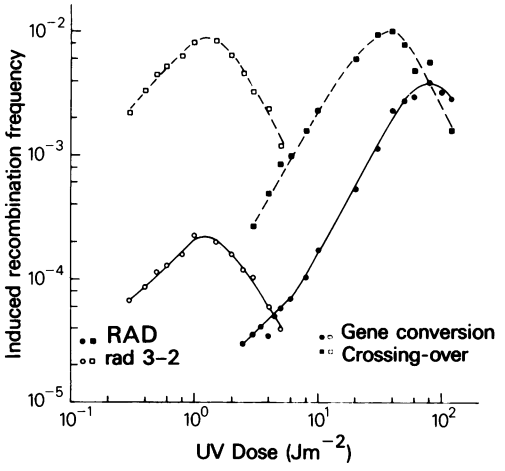


**Figure 3** UV-induced mitotic recombination, measured as gene conversion at the *TRP* 5 locus and mitotic crossing-over at the *ADE* 2 locus in diploid strains of yeast (Kunz & Haynes, 1981), in the dimer-repair deficient strain *rad* 3-2 and in its *RAD* wild-type parent. Note that despite the complexity of these curves, in the low dose range the frequencies for *rad* 3-2 are substantially greater than those for Rad wild-type. This indicates that pre-recombinagenic damage is not repaired in *rad* 3-2 whereas it is in RAD. The high dose declines in frequency may arise from a number of sources, but formally are equivalent to "$\delta$-effects" for which the probability of recombinant clone formation is less than that for non-recombinants.

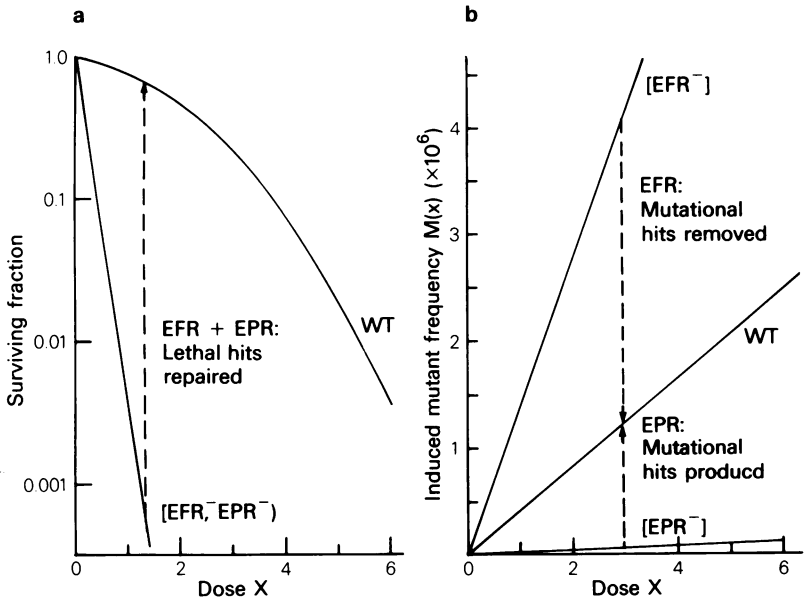


**Figure 4** Schematic illustration of the DNA damage-repair concept. (a) Typical UV survival curve for normal, repair proficient wild-type cells together with that for a mutant deficient both in error-prone and error-free modes of repair. The shoulder and increasing (negative) slope of the wild-type reflect the decline in repair efficiency with increasing dose; because of the absence of repair, no such shoulder appears on the mutant curve. The difference in log survival levels between these two curves for any given dose gives the number of lethal hits removed by repair. (b) Typical UV-induced mutation frequency curve for repair-proficient cells (WT) together with those for two UV-sensitive mutants, one deficient in error-free repair ($EFR^-$) and the other deficient in error-prone repair ($EPR^-$). For stochastically independent mutation and killing events, the difference between $EFR^-$ and WT for any given dose is equal to the number of mutational hits removed by error-free repair, whereas the difference between WT and $EPR^-$ is equal to the number of mutational hits produced by error-prone repair. Note also that in this diagram, the dose-mutation curves are plotted on rectilinear scales; in practice, such curves are often plotted on double-log scales as in **Figures 2 and 3**.

repair capacity. In at least one case the difference both in shape and sensitivity between two such curves, for UV-irradiated haploid yeast, has been shown to agree quantitatively with biochemical measurements of the efficiency of pyrimidine dimer excision as a function of dose (Wheatcroft *et al.*, 1975). At any given dose, the vertical difference between the two curves is proportional to the number of lethal hits removed, or bypassed, by repair systems that are present in the wild-type strain, but missing or deficient in the mutant. The shoulder on the wild-type curve thus reflects a decline in repair efficiency with increasing dose. This decline could be caused *either* by excessive DNA substrate damage at high doses, *or* by inactivation of the enzymes involved in repair; it is impossible to distinguish between these two alternatives for declining repair efficiencies on the basis of survival curve analysis alone.

Repair systems can act also on pre-mutational and pre-recombinagenic lesions in DNA. Thus, mutants defective in repair can exhibit elevated levels of mutagenesis and mitotic recombination, as illustrated in Figures 2 and 3, and shown schematically by the upper line in the right-hand panel of Figure 4. However, there also exist radiation and chemical-sensitive mutants of yeast, and other organisms, that show greatly *depressed* levels of induced mutation (and mitotic recombination) (Haynes & Kunz, 1981). Such suppression of mutability accompanied by enhanced sensitivity to killing, caused by single mutant genes, led Witkin (1969) to distinguish between two categories of repair called "error-free" and "error-prone". The bottom line in the right-hand panel of Figure 4 indicates the mutational response of a mutant strain deficient in error-prone repair. On this basis, repair systems are seen to be involved as causative, as well as ameliorative, factors in radiation and chemical-induced mutagenesis. The observed levels of induced mutagenesis in repair proficient wild-type strains result from the interplay of error-free and error-prone repair processes acting on pre-mutational lesions in DNA (middle line,

right-hand panel, Figure 4). An analogous situation exists also for induced mitotic recombination, and indeed, recombination is coming to be regarded evolutionarily as a repair process, rather than a generator of genetic diversity.

It is important to realize that the terms "error-free", "error-prone" and "recombinagenic" repair refer only to *operational* concepts and not to specific enzymic processes. At the biochemical level, a given repair system could, in principle, be either error-prone or error-free. Excision repair of pyrimidine dimers generally is error-free, but there are some circumstances, for example in thymidylate-starved yeast, where its presence may contribute to genetic change. In bacteria, such as *Escherichia coli,* what is believed to be a major mechanism of "error-prone repair", the so-called "SOS" response (Witkin, 1976), involves the *bypass,* rather than the actual removal or reversal of DNA damage and so, strictly speaking, does not constitute repair at the molecular level. Thus, terminology in this area must be used and interpreted with caution.

If one is to analyze dose-response relations quantitatively, these qualitative ideas must be given mathematical expression. We do this in three sequential steps. The first, most abstract, step constitutes a purely formal description of dose-response curves obtained simply from application of single-event Poisson statistics to the phenomena of cell killing and mutagenesis. The second consists in writing general expressions for the number of lethal or mutational hits in terms of the DNA damage-repair hypothesis. It is at this point that mechanistic assumptions first enter the mathematical development. Finally, the repair efficiency functions can be rendered more explicit by introducing a variety of specific assumptions regarding the induction and inactivation of error-free and error-prone repair.

*Step 1: Formal description of dose-response relations*

It is useful to distinguish formal, stochastic, descriptions of dose-response relations from mathematical models of the underlying molecular mechanisms. Purely formal analysis of radiobiological data has been used to identify, by empirical curve-fitting, various kinetic response patterns for killing, mutation and mitotic recombination (Haynes & Eckardt, 1980), to classify radiation- and chemical-sensitive mutants into distinct "epistasis" groups (Haynes & Kunz, 1981), to quantify relative mutagenic efficiencies of various mutagens and mutational sensitivities of various organisms (Eckardt & Haynes, 1980), to detect inducible components in mutation frequency curves, and to demonstrate stochastic dependence of killing and mutation in certain strains of yeast (Eckardt & Haynes, 1977). We develop the necessary formalism by applying single-event Poisson statistics to typical experimental protocols for the measurement of cell survival and mutagenesis in suspensions of irradiated cells.

Consider a homogeneous suspension of $N_0$ single, equally sensitive cells per unit volume that is uniformly treated with various exposure doses $x$ of some mutagen. After each dose the number of surviving cells, $N_s(x)$ and the number of induced mutants (or recombinants) among these survivors, $N_m(x)$, are scored in suitable assay systems. Two basic biological quantities can be measured: the surviving fraction of cells, $S(x)=N_s(x)/N_0$, and the induced mutant yield, $Y(x)=N_m(x)/N_0$ (mutants per initial viable cell). Mutation frequency (mutants per survivor) is calculated from these measurements as $M(x)=N_m(x)/N_s(x)=Y(x)/S(x)$. Thus we are interested in three sorts of dose-response functions, $S(x)$, $Y(x)$ and $M(x)$ (Haynes & Eckardt, 1980).

We make two statistical assumptions. First, that the all-or-none character of the measured biological endpoints (survivor *vs* non-survivor, mutant *vs* non-mutant) allows the application of single-event Poisson statistics to the processes of killing and mutagenesis. Second, that mutation and killing are stochastically independent processes in the sense that the probability of macrocolony formation after a mutagen dose $x$ is the same for both mutated and non-mutated cells.

We denote the average, or expected number of lethal and mutational hits per cell in the population by the functions $H_k(x)$ and $H_m(x)$, respectively. Since, insofar as can be determined experimentally, it is a matter of chance which cell dies, or survives and is mutated, we consider these biological hits as being distributed randomly in the population. If a cell sustains a single lethal hit, by definition, it is dead, even though we do not specify the biochemical causes of death. Thus, on the basis of single-event Poisson statistics, the probability of survival is $\exp[-H_k(x)]$ and the surviving fraction of cells can be written as

$$S(x)=e^{-H_k(x)} \tag{1}$$

Similarly, if a cell sustains a mutational hit, but no lethal hit, it will survive as a mutant. The mutant yield is the joint probability of these two events and is therefore,

$$Y(x)=[1-e^{-H_m(x)}]\,e^{-H_k(x)} \simeq H_m(x)e^{-H_k(x)} \tag{2}$$

By definition, the mutation frequency is given by the ratio of (2) to (1) and so we have

$$M(x)=[1-e^{-H_m(x)}] \simeq H_m(x) \tag{3}$$

The approximations in equations (2) and (3) are valid since mutation frequencies are very much less than unity. In writing equation (2) it is assumed that the probability of survival of the mutants is the same as that for cells in the population as a whole. If this is not the case, then mutation and killing are not stochastically independent processes and we say there are "$\delta$-effects" in the population (Eckardt & Haynes, 1977; Haynes & Eckardt, 1980). Further discussion of this phenomenon is beyond the scope of the present paper.

Lethal hits are defined biologically on the basis of equation (1): $H_k(x)$ hits are said to have occurred at a dose that leaves a fraction $\exp[-H_k(x)]$ of cells as survivors in the assay system employed. Clearly, the number of lethal hits is given by $-lnS(x)$ and so is proportional to ordinate distances in semi-log plots of surviving fraction. If the relevant physical lesions are formed in direct proportion to dose, and if no dose-dependent processes are involved in the conversion of these lesions into biological hits, then the hit function will be linear as in classical single-hit theory. Furthermore, in this case the number of lethal hits is proportional to the number of initial lesions for any dose. However, in general, $H_k(x)$ may be considered a non-linear function of dose. Similar considerations apply to $H_m(x)$.

In classical hit theory, the Poisson distribution is used to calculate the probability that no *physical* hit occurs in a cell; thus, it is not unreasonable to entertain the idea of multiple hits being required for cell death. In repair theory, the Poisson distribution is used to calculate the probability that no *biological* hit occurs in a cell; on this basis it is unreasonable to imagine that multiple lethal hits would be required for cell death.

Theoretically, it is possible to imagine that more than one Poisson process is involved in cell killing or mutation. If this view is adopted then equations (1) and (3) would be written as products of independent exponential terms, one for each assumed process (see, for example, Bender & Gooch, 1962). However, we find such approaches objectionable since, as mentioned earlier, it is only lethal or mutational hits that are *observed* to be randomly distributed among the cells of the population. Various unobserved stochastic processes undoubtedly occur at the macromolecular level, but assumptions regarding them are mechanistic in nature and are appropriately introduced in mechanistic expressions for the biological hit functions $H_k(x)$ or $H_m(x)$.

It is useful to express the biological hit functions defined in equations 1–3 as infinite series. Any well-behaved function can be written as a power series, thus we have,

$$-lnS(x)=H_k(x)=k_1x+k_2x^2+k_3x^3+ \quad (4)$$

and

$$M(x)=H_m(x)=m_1x+m_2x^2+m_3x^3+ \quad (5)$$

These series can be truncated to various finite polynomials that are useful for empirical curve fitting,* and in studies of the properties of survival, and mutation yield and frequency curves, for various kinetic response patterns. For example, a purely "linear" response pattern is one in which all $k_i$ and $m_i$ are zero except for $k_1$ and $m_1$. In this case the survival curve is exponential, the mutation frequency curve is linear with slope $m_1$, the mutant yield curve has a slope $m_1$ at the origin and rises to a maximum at the 37% survival level from whence it declines at a rate governed by the value of $k_1$. The initial slope of the survival curve may be zero or non-zero depending on the value of $k_1$ which might be expected to vary from one cell type to another. The final slope is infinite for any finite polynomial approximation of the hit functions, but is finite if the infinite series represents some function that is well-behaved asymptotically. The dose-response curves for more complex kinetic patterns also have been worked out and published recently (Haynes & Eckardt, 1980).

*Step II: Mechanistic repair models*

The second step of our analysis consists in expressing the biological hit functions in mechanistic form. It is at this stage in the analysis that specific assumptions regarding the underlying molecular mechanisms are first made.

Within the context of the formalism developed above it is possible to imagine a variety of mechanistic models, each focussed on different aspects of the complex cellular and molecular processes involved in killing and mutagenesis.

*Prof. H.D. Mennigmann (Frankfurt), using his extensive UV-survival and mutation data for *E. coli*, has worked out values of the coefficients $k_i$ and $m_i$ for various finite polynomials. Maximum likelihood methods can be used to assess the goodness-of-fit of the polynomials to the data. While some simple models can be ruled out on this basis, in general, it is impossible to establish the validity of any particular model by curve-fitting alone. Furthermore, as the number of terms increases, the values of the coefficients do not converge rapidly and it is not possible to determine the relative magnitudes of, say, the linear and quadratic terms in the expansion. A more satisfactory numerical approach, which so far has not been used for radiobiological data, would be to approximate the hit-functions as series of Chebyshev polynomials. In this way optimal approximation can be achieved with a minimal number of terms, and the error in the approximation is uniformly distributed over the range of the data.

However, the viewpoint adopted for the construction of any such model should be constrained by available biochemical, genetic and physiological concepts.

We begin by asking just what, at the molecular level, a lethal or mutagenic hit might be? On the basis of the DNA damage-repair hypothesis we assume that a lethal hit corresponds to one (or more) potentially lethal lesion in DNA that remains permanently unrepaired by any of the cell's repair mechanisms. As in classical hit theory, no assumptions are made regarding the actual mechanisms of death or mutation. Thus, in the simplest case of one class of potentially lethal lesions and a single mechanism, or pathway, for their repair, we can write,

$$-lnS(x) = H_k(x) = F_k(x)\,[1-r(x)] = F_k(x) - R(x) \quad (6)$$

where $F_k(x)$ is the number of potentially lethal lesions produced by dose $x$ and $r(x)$ is the efficiency with which they are repaired at dose $x$. $r(x)$ is defined and measured as the ratio of the number of lesions repaired to the number initially formed. $[1-r(x)]$ is the probability that a lesion is *not* repaired. $R(x)$ is the number of lesions removed by repair at dose $x$. Equation (6) sets out both the definition of lethal hits in terms of their assumed biological realization as unrepaired lesions in DNA. Both $F_k(x)$ and $r(x)$ can be measured experimentally and so, in principle, it should be possible to predict survival data from such measurements; this has been done in one case of UV-irradiated hoploid yeast (Wheatcroft *et al.*, 1975). Equation (6) thus links the formal definition of lethal hits with one particular mechanistic model.

More generally, if a cell possesses several pathways for repair, then additional terms of the form $[1-r(x)]$ would be used to multiply $F_k(x)$. Furthermore, if there were several distinct categories of potentially lethal, but repairable, lesions, each acted upon by different systems, then $F_k(x)$ would have to be broken out into a sum of terms for each category, and each of these would be multiplied by appropriate repair efficiency functions. There might also exist lesions that are intrinsically unrepairable; the $F_k(x)$ term for this category obviously would not be multiplied by any repair function (Brendel & Haynes, 1973; Haynes, 1975).

In experiments in which both mutation and killing are measured, it becomes necessary to distinguish explicitly between error-free and error-prone modes of repair (cf., Figure 4). We do this mathematically by writing the expected number of lethal hits at dose $x$ in the form,

$$H_k(x) = F_k(x)[1-r(x)][1-r_m(x)] \quad (7)$$

where $r(x)$ now is taken to mean the efficiency of error-free modes of repair, and $r_m(x)$ is the efficiency of error-prone modes of repair. Similarly, the expected number of mutational hits can be written,

$$H_m(x) = F_m(x)[1-r(x)]r_m(x) \quad (8)$$

where $F_m(x)$ is the number of pre-mutational lesions initially formed. Equation (8) expresses the basic assumption of the repair model for mutagenesis: mutational hits are lesions that escape error-free repair but *are* processed by error-prone repair. Equations (7) and (8) together constitute a mathematical representation of the DNA damage-repair hypothesis.

*Step III: Explicit representation of lesion and repair functions*

The third stage of analysis involves writing explicit expressions for the initial lesion and repair functions that occur in equations (6), (7) and (8). Unfortunately, this must be done at present on an intuitive basis. We have very little experimental information to help us here.

The forms of the $F_k(x)$ and $F_m(x)$ functions are relatively easy to adduce. It is known by direct measurement that the numbers of various mutagen-induced lesions in DNA generally increase in direct proportion to dose in the biologically significant range. This applies, for example, to UV-induced pyrimidine dimers and X-ray induced single stand breaks. Thus, $F_k(x) = Kx$ often is an adequate representation of the lesion function. In cases where there is reasonable evidence for the involvement of "two-hit" lesions, such as double strand breaks arising from two closely spaced single breaks, then the linear-quadratic form, $F_k(x) = K_1x + K_2x^2$ becomes appropriate.

The first attempt to arrive at an explicit representation of a repair function was based on the idea that the number of lesions repaired should increase linearly at low doses, but that a cell's capacity for repair would become saturated at sufficiently high doses (Haynes, 1966). Thus, a simple saturation function was proposed to describe the dose-dependence of the number of lesions repaired. In equation (6) we would write, therefore,

$$R(x) = \alpha[1-e^{-\beta x}] \quad (9)$$

where $\alpha$ and $\beta$ represent respectively, the maximum number of potentially lethal hits that can be repaired, and the rate of saturation of the repair system with increasing dose. This formula, together with a linear representation of $F_k(x)$, leads to curves that provide good fits to UV-survival data for *E.*

*coli* (Haynes, 1966). In addition, this function possesses certain theoretically desirable features, such as the possibility for both zero and non-zero initial slopes, and finite final slopes, for survival curves (Alper, 1979).

Wheatcroft's measurements of dimer excision efficiency indicated that the *number* of dimers excised in UV-irradiated yeast did indeed increase linearly at low doses, but at high doses reached a maximum and then declined. Thus, a saturation function in the form of equation (9) would be inappropriate for such a case (Wheatcroft *et al.*, 1975), Direct plots of the dose dependence of excision efficiency indicated that the standard 2-target equation provided a good representation of the data, that is,

$$r(x) = 1 - [1 - e^{-\beta x}]^2 \qquad (10)$$

For such data the meaning of target multiplicity is not clear, although again, survival curves of acceptable shape are generated by a reasonable repair function.

Still more general expressions for repair can be constructed by taking account of the fact that there exist inducible as well as constitutive components of error-free and error-prone repair, the efficiencies of which may be reduced at high doses as suggested above. On this basis, $r(x)$ and $r_m(x)$ in equation (7) and (8) can be written in the form,

$$r(x) = [r_c + r_\sigma U(x)] V(x) \qquad (11)$$

$$r_m(x) = [r_{mc} + r_{m\sigma} U_m(x)] V_m(x) \qquad (12)$$

where $r_c$ and $r_{mc}$ are the initial efficiencies of the *constitutive* components of error-free and error-prone repair, respectively; and $r_\sigma$ *and* $r_{m\sigma}$ are the maximum repair efficiencies of the *induced* components of error-free and error-prone repair. $U(x)$ and $U_m(x)$ represent the induction kinetics for the inducible components, and $V(x)$ and $V_m(x)$ represent the inactivation of both constitutive and inducible components. If the $U(x)$ terms are assumed to be exponential saturation functions, and the $V(x)$ terms exponential decay functions then it is possible to substitute these expressions in equations (7) and (8), expand them in infinite series, and thereby obtain expressions for the $k_i$ and $m_i$ coefficients in the empirical representation of the hit function (equations 4 and 5). In this way it is possible to interpret those empirical curve parameters in terms of the DNA damage-repair hypothesis (Haynes & Eckhardt, 1980). However, application of the more complex functions of the sort illustrated in equations (11) and (12) has only just begun.

## Conclusions

The repair theory for the interpretation of radiobiological dose-response data is based on much the same statistical approach as classical single-hit theory. However, in repair theory, a clear distinction is made between formal and mechanistic dose-response equations. Furthermore, the biologically significant hits are not identified with initial radiation absorption events. Rather, it is assumed that cell killing is caused by lesions in DNA that escape both error-free and error-prone modes of repair, while mutational events arise from lesions that escape error-free repair, but are repaired, or bypassed, by error-prone processes. The concept of "sublethal" damage is not invoked. Repair theory can be applied readily to cell killing and mutation by chemical mutagens and ultraviolet light, as well as ionizing radiation, since hits are not identified with ionization events.

Shouldered survival curves are better fitted by repair models than by models of the multitarget/hit type since the latter predict zero initial slopes, which are by no means an obvious or even common feature of radiobiological dose-response patterns. There is extensive biochemical evidence for the existence of several modes of enzymic repair of UV, X-ray and chemical mutagen damage to DNA in cells. Studies on mutants defective in these repair processes have made it possible to identify important mechanisms responsible for radio-resistance, and certain recovery phenomena, observed at the cellular level. It now seems clear that the classical hit and target theories are no longer useful guides to experiment in radiation biology.

Perhaps because of the increasingly obvious difficulties with classical hit theory, it has become almost fashionable to claim that nothing can be learned from the analysis of dose-response relations for the genetic effects of radiations and chemical mutagens. However, despite the truism that explicit molecular mechanisms cannot be deduced from survival or mutation frequency curves, mathematical analysis does compel one, at least, to make explicit the assumptions and ideas used to describe the phenomena. Even more important is the heuristic power of such analysis in guiding those biochemical experiments that can illuminate the macromolecular processes involved. The mathematical work reviewed here has already been of considerable assistance in analyzing the genetic effects of ultraviolet light and other mutagens on microorganisms.

Having just sung the merits of repair theory, it is ironic now to point out that evidence already is increasing for a much more complex biochemical

picture of cellular responses to radiation and chemical mutagens than that provided by the DNA damage-repair hypothesis. Work in a variety of *in vivo* and *in vitro* systems has shown that disturbances in pool sizes of the deoxyribonucleotide precursors of DNA synthesis can have profound genetic consequences. *In vivo* effects include cell killing, mutation, mitotic recombination, chromosome aberrations, aneuploidy, prophage induction, oncogenic transformation, teratogenesis, and DNA strand breaks (Kunz, 1982). Both deoxyribonucleotide pool imbalances, and this familiar range of presumably related biological effects, can be produced by agents such as antifolate drugs and nucleotide analogs, that do not attack DNA but rather inhibit specific enzymes involved in nucelotide biosynthesis. Thus, it is clear that cells contain important non-DNA primary targets for the induction of genetic change, at least by various anti-cancer drugs (Haynes *et al.*, 1982). Furthermore, it has been shown recently that attack on cells by certain standard mutagens, such as ultraviolet light and nitrosoguanidine, leads to expansion of dATP and dTTP pools in both bacteria and mammalian cells (L. Loeb & T. Kato, personal communication). The time seems ripe for the development of more sophisticated biochemical models of mutagen actions than that provided by the simple DNA damage-repair hypothesis.

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